Application Note

Channel: nACha7R
Cells: HEK293
Tools: Patchliner

Nicotinic ACha7R activation and pharmacology recorded on Nanion's Patchliner

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Cells kindly provided by Galantos Pharma, GmbH, Germany.

Summary

The nicotinic acetylcholine receptor (nAChR) is a member of the ligand-gated ion channel superfamily which includes $GABA_A$, $5HT_3$, NMDA and glycine receptors. It is a cation-permeable ion channel activated by the neurotransmitter acetylcholine and the natural alkaloid, nicotine. Neuronal nAChR are pentameric and functional channels are formed from a repertoire of nine a (a2 to a10) and three β subunits (β2 to β4). Most nAChR exist as heteromers with the stoichiometry 2a to 3β, however some a subunits function as homomers, these being a7 or a9 (for reviews see Refs. 1 & 2). nAChR have been proposed to play a role in many neurological disorders such as Alzheimer's Disease, Parkinson's, schizophrenia and depression[1-4]. nACha7R are widely distributed in the mammalian brain including in the cerebral cortex, hippocampus, basal ganglia and cerebellum[4]. There is evidence that nACha7R play a role in cognition[5,6] and could be a potential therapeutic target in cognitive disorders such as Alzheimer's Disease or schizophrenia[3,5,6].

Here we present data collected on a Patchliner showing the potential use of the Patchliner to record fast desensitizing nACha7R currents activated by acetylcholine (ACh) or nicotine. ACh and nicotine activated nACha7R in a concentration-dependent manner with an EC_{50} similar to those reported in the literature[7-10]. nACha7R could be repetitively activated by nicotine and were enhanced by the Type I positive allosteric modulator (PAM), NS1738, with an EC_{50} in good agreement with the literature[11].

Results

Nicotinic ACha7R were activated by increasing concentrations of ACh or nicotine (Figure 1). A concentration response curve for ACh (Fig. 1B) revealed an EC_{50} of 304 ± 42 µM (n = 11) in good agreement with the range found in the literature[8-10]. Raw traces from an example cell are shown in Fig. 1A. The concentration response curve for nicotine is shown in Fig. 1C which reveals an EC_{50} = 70 ± 14 µM (n = 3), in good agreement with the literature[7-9].

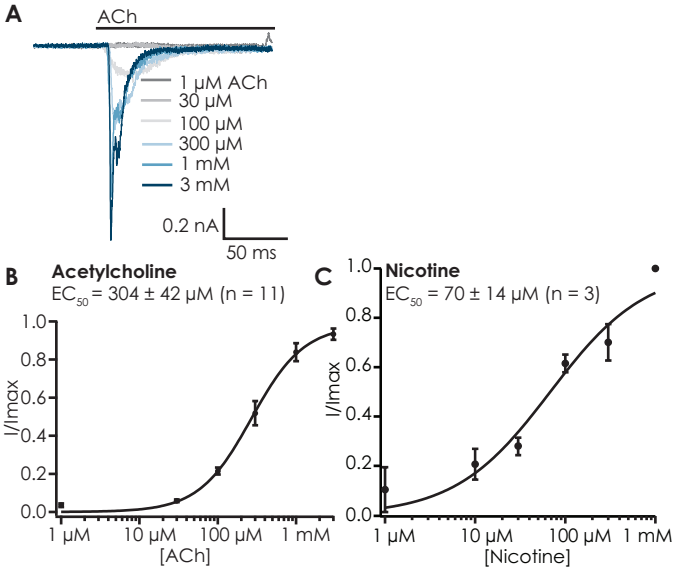

Figure 1: Activation of nACha7R by increasing concentrations of ACh or nicotine. **A** Raw traces from an example cell showing activation of nACha7R by increasing concentrations of ACh. **B** Concentration response curve for ACh activation for an average of 11 cells, EC_{50} = 304 ± 42 µM (n = 11). **C** Average concentration response curve for nicotine activation, EC_{50} = 70 ± 14 µM (n = 3).

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Figure 2 shows the repetitive activation of nACha7R. Currents were activated with a similar peak amplitude when challenged 8 times with 100 µM nicotine.

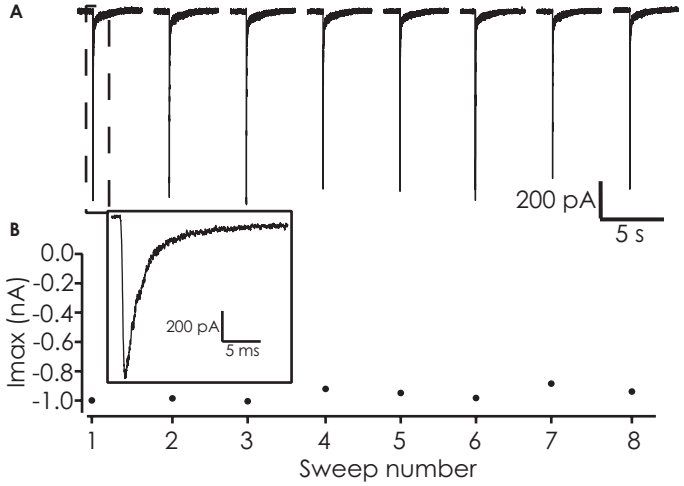

Figure 2: A Nicotinic ACha7R could be repetitively activated by 100 µM nicotine. Inset shows nACha7R activation of the first application expanded **B** Timecourse of the experiment. Nicotinic ACha7R currents of approx. 1 nA were activated 8 times reproducibly in the same cell.

NS1738 is a Type I PAM of nACha7R which increases amplitude without any profound effects on desensitization[11]. It has been shown to produce cognitive enhancement in rats. We show that NS1738 enhanced nACha7R-mediated responses when pre-incubated and then co-applied with acetylcholine (Fig. 3). A full concentration response curve to NS1738 was performed revealing an EC_{50} of 2.6 ± 1.1 µM (n = 4), in excellent agreement with the literature[11].

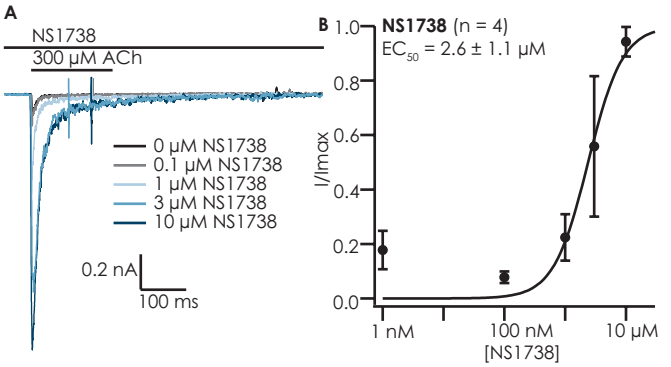

Figure 3: Enhancement of nACha7R by NS1738. NS1738 at increasing concentrations (0.1 µM - 10 µM) was pre-incubated and then co-applied with 300 µM ACh. NS1738 enhanced the peak of the ACh response at a concentration of 1 µM or above. **B** Concentration response curve for NS1738 enhancement, EC_{50} = 2.6 ± 1.1 µM (n = 4).

In summary, nACha7R stably expressed in HEK293 cells can be reliably activated by nicotine or ACh and enhanced by the Type I PAM, NS1738, with EC_{50} values in good agreement with published literature[7-11]. Therefore, the Patchliner provides a viable, higher throughput alternative to conventional patch clamp for the discovery of agonists of nACha7R as potential therapeutics to enhance cognitive function.

References

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Methods

Cells

An inducible HEK293 cells stably expressing human nACha7R were used.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner. Cells were held at a holding potential of -80 mV. To achieve short exposure times, solutions were stacked in the robotic pipettor. First, wash solution (280 µl) was aspirated followed by aspiration of the agonist-containing solution (30 µl) and then rapidly applied to the cell at a speed of 171 µl/s. Wash solutions contained the PAM in the NS1738 experiments. The cells were pre-incubated in NS1738 before co-application with 300 µM ACh.

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